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**THE PRESENCE OF AGGLUTININS FOR
BRUCELLA ABORTUS IN MILK****BULLETIN NO. 369**

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The Presence of Agglutinins for *Brucella Abortus* in Milk

By H. B. MORRISON and F. E. HULL

A few years ago an investigation was undertaken on milk from cows in the Kentucky Agricultural Experiment Station dairy herd which, at that time, contained both Bang's disease positive and negative cows. One of the tests made was the determination of the presence of agglutinins for *Brucella abortus* in individual milk samples from each quarter of the udder. Later, an opportunity was offered to continue this study in two large commercial dairy herds in Kentucky. The data in this paper are taken from the results obtained from these three herds.

CONDITIONS OF HERD MANAGEMENT

In Herd No. 1 the Bang's disease positive cows and Bang's disease negative cows were pastured in separate pastures and housed in separate barns about two hundred yards apart. The two groups were milked by hand and cared for by separate crews of men and thus had no contact with each other. The positive cows were mainly older cows which were kept largely because of their value as breeding stock.

Herd No. 2, while on one farm, was kept in two units designated as 2a and 2b, as they were located more than a mile apart and the system of management was considerably different. In Herd No. 2a, the positive and negative cows were kept in separate pastures but housed in the same barn. They entered the barn by different doors and a feed alley separated the positive and negative cows. All these cows were milked by hand by the same group of men. The cows in Herd 2b were pastured

and housed together. No attempt was made to keep them separated in the barn.

The positive and negative cows in Herd No. 3 used separate pastures but were housed in the same barn. They were kept together in groups in the barn with at least one vacant stall separating the positive and negative animals. One group of men handled both groups. Milking was done by machine, the same machines being used for both groups but the negative cows were milked first. The number of cows in each herd and the number of samples taken are shown in Table 1.

Table 1. Number of samples from individual quarters of the udder and number of cows from which samples were taken.

	Herd 1	Herd 2a	Herd 2b	Herd 3	All Herds
Positive Cows					
Number	51	9	27	12	99
Samples	197	35	100	116	448
Negative Cows					
Number	32	44	21	35	132
Samples	126	173	79	321	699
Total Cows					
Number	83	53	48	47	231
Samples	323	208	179	437	1,147

PROCEDURE

Milk samples were taken aseptically from each quarter of each cow's udder. The udder was washed and dried, and a few streams were milked from each teat and discarded. The milk for the samples was then collected in sterile glass containers. Samples for the agglutination test were later transferred to small test tubes containing approximately 0.01 gram of powdered rennet. The serum which exuded from the clot was used in the agglutination test. Determinations were made at a dilution of 1 to 50 by the rapid method using Huddleson *Brucella abortus* antigen. Agglutination reactions were recorded as negative, +, ++, +++, and complete. For the sake of brevity, the

+, ++, and +++ reactions are included under the heading, partial agglutination, in all tables except Table 7. The cows were classed as Bang's disease positive or negative according to the results of the latest blood agglutination test previous to the collection of the milk samples.

RESULTS

As might be expected in several herds under different systems of management, there was considerable difference in the percentage of milk samples from Bang's disease positive cows giving a positive agglutination reaction. As is indicated in Table 2 the percentage of samples giving partial and complete

Table 2. Distribution of agglutination in milk from cows in four herds, as percent of the number of samples tested.

	Herd 1 Percent	Herd 2a Percent	Herd 2b Percent	Herd 3 Percent	All Herds Percent
Positive Cows					
No agglutination	43.7	45.7	60.0	56.0	50.7
Partial agglutination ..	13.2	37.2	21.0	23.3	19.4
Complete agglutination ..	43.1	17.1	19.0	20.7	29.9
Negative Cows					
No agglutination	100	100	98.7	100	99.9
Partial agglutination ..	0	0	1.3	0	.1
Complete agglutination ..	0	0	0	0	0

agglutinations varied from 40.0 in Herd 2b to 56.3 in Herd 1, while the average for samples from all herds was 49.3 percent. In the samples from three of the groups there was a larger percentage of partial than of complete agglutinations at 1 to 50. However, because of the large majority of complete agglutinations in Herd 1, which was the largest herd, the complete agglutinations outnumbered the partial ones about three to two for the entire group. The larger percentage of complete agglutinations in this one herd may have been because it was composed of old cows which had been reactors for a longer time than the

others and so had more chance for their udders to become infected. Of six hundred and ninety-nine milk samples from Bang's disease negative cows only one (0.14 percent) gave any

Table 3. Agglutination reaction of milk from each quarter of the udder of Bang's disease positive cows.

Herd	Quarter of Udder	No. of Samples	Reaction		
			Negative	Partial	Complete
			Percent	Percent	Percent
1	LF	49	44.9	16.3	38.8
	LH	49	44.9	10.2	44.9
	RF	51	43.1	15.7	41.2
	RH	48	41.7	10.4	47.9
	All	197	43.7	13.2	43.1
2a	LF	9	55.6	33.3	11.1
	LH	8	50.0	25.0	25.0
	RF	9	44.5	33.3	22.2
	RH	9	33.3	55.6	11.1
	All	35	45.7	37.2	17.1
2b	LF	25	48.0	36.0	16.0
	LH	25	60.0	16.0	24.0
	RF	26	61.5	23.1	15.4
	RH	24	70.9	8.3	20.8
	All	100	60.0	21.0	19.0
3	LF	29	65.5	20.7	13.8
	LH	29	48.3	24.1	27.6
	RF	29	62.1	20.7	17.2
	RH	29	48.3	27.6	24.1
	All	116	56.0	23.3	20.7
All Herds	LF	112	51.8	23.2	25.0
	LH	111	49.5	16.2	34.2
	RF	115	52.2	20.0	27.8
	RH	110	49.1	18.2	32.7
	All	448	50.7	19.4	29.9

agglutination and in this instance agglutination was only partial.

When all the agglutination reactions are tabulated according to the quarter of the udder sampled (Table 3), there is about the same percentage of positive and negative reactions from each quarter. However, in three of the four herds there was considerable variation in this respect. In one herd the left front quarter and in another the right hind quarter showed the highest percentage of positive agglutinations. One herd gave about the same percentage of positive agglutinations for both hind quarters, the percentages from these quarters being higher than those from the corresponding front quarters, while the percentages of positive reactions were about the same from all quarters of the cows in the other herd. The left and right halves gave almost exactly equal percentages of positive agglutinations while the hind quarters gave a slightly higher percentage of positive reactions than did the front quarters.

A considerable difference was found in the agglutinin titer of milk from different quarters of the same cow's udder, as is indicated in Table 4. About one-third (36.2 percent) of the

Table 4. Distribution of agglutination in milk from individual quarters of the udders of positive cows.

	Herd 1	Herd 2a	Herd 2b	Herd 3	All Herds	Percent
All negative	16	1	12	13	42	36.2
Negative and partial	8	4	7	2	21	18.1
Negative, partial, and complete	3	1	1	4	9	7.8
Negative and complete	1	1	0	2	4	3.4
All partial	1	0	0	5	6	5.2
Partial and complete	5	2	5	0	12	10.3
All complete	17	0	2	3	22	19.0
Number of cows	51	9	27	29	116	100.0

Bang's disease positive cows showed no milk agglutination in any quarter of the udder, while nearly two-thirds (63.8 percent) gave at least partial milk agglutination in one or more quarters. The largest group of these (19.0 percent) showed complete agglutination in all quarters, followed closely by a group (18.1 percent) which gave negative and partial agglutinations. Other groups in order of size were those giving partial and complete (10.3 percent), negative, partial and complete (7.8 percent), all partial (5.2 percent) and negative and complete agglutinations (3.4 percent).

The number of cows giving negative agglutinations only, those with some partial but no complete agglutinations and those showing complete agglutination in one or more quarters is shown in Table 5. The duplicates, i.e., where more than one set of

Table 5. Agglutination reactions of milk from positive cows, showing number of cows with low and high titers.

	Herd 1	Herd 2a	Herd 2b	Herd 3	All Herds	Percent
All quarters negative	16	1	12	6	35	35.4
One or more quarters partial but none complete	9	4	7	0	20	20.2
One or more quarters complete	26	4	8	6	44	44.4
Number of cows	51	9	27	12	99	100.0

samples were taken from the same cow (in Herd 3) have been eliminated. In the entire group of cows about one-third gave no milk agglutination in any quarter while nearly one-half showed complete agglutination in one or more quarters. About twice as many cows showed complete milk agglutination as gave no higher than partial agglutination in any quarter.

Some idea as to the extent of the occurrence of agglutinins

thruout the udder is given in Table 6. Of the cows whose milk showed any agglutination, more than half showed agglutination

Table 6. Distribution of agglutination in milk from individual quarters of the udders of positive cows showing the extent to which the udder is involved.

	Herd 1	Herd 2a	Herd 2b	Herd 3	All Herds	Percent
All quarters negative -----	16	1	12	13	42	36.2
1 quarter positive -----	6	2	3	2	13	11.2
2 quarters positive -----	1	2	3	1	7	6.0
3 quarters positive -----	6	2	3	5	16	13.8
All quarters positive -----	22 ^{1,2}	2 ²	6 ³	8	38	32.8
Number of cows	51	9	27	29	116	100.0

¹ One cow in this group had 2 blind quarters.

² One cow in this group had 1 blind quarter.

³ Two cows in this group had 1 blind quarter.

in all the quarters giving milk, while the rest showed agglutination in milk from one, two or three quarters with at least one quarter negative. In only one case out of thirteen where agglutinins were demonstrated in milk from only one quarter was the agglutination complete. Approximately the same was true in cows having two quarters showing agglutination. By far the largest number of complete agglutinations occurred in cows whose milk from all quarters was agglutinated.

In nine of the thirteen cases where only one quarter was involved, agglutination was found in samples from hind quarters. The cases where milk from only one quarter was found negative were about equally distributed among the different quarters of the udder.

Two or more sets of samples were taken from some of the

cows in Herd No. 3. The results from these tests (Table 7) indicate the variability of the agglutinin titer of milk at different times.

Table 7. Agglutination reactions of milk taken at different times from Bang's disease positive cows.

Cow	Date	Reaction ¹			
		LF	LH	RF	RH
E 57 H	10/6/31	c	c	—	++
	11/16/31	—	c	c	c
	9/16/32	+++	++	++	+++
	10/4/32	++	++	+	++
	10/15/32 ²	—	—	—	—
	12/7/32	+	+++	+	+
E 27 H	10/6/31	—	++	—	—
	11/17/31	—	—	—	+
	10/4/32	c	c	c	c
	12/7/32	+	+++	+	+++
E 8 H	10/21/31	—	—	—	—
	11/16/31	—	—	—	—
	9/14/32	—	—	—	—
E 22 J	10/13/31	—	c	c	++
	9/14/32	+	++	—	c
	12/7/32	++	++	++	++
E 69 H	10/19/31	—	c	++	c
	11/17/31	—	c	—	c

¹—, negative; +, ++, +++, partial; c, complete.

² 10/15/32 Milk from individual quarters of E 57 was injected into guinea pigs. Milk from left hind quarter caused guinea pig to abort. Fetuses cultured — negative. Guinea pig autopsied and cultured — organism recovered.

Inoculation tests by Miss Helen Harms, Assistant in Animal Nutrition.

times. In some instances, milk from one quarter of the udder showed no agglutination, while samples taken at other times from the same quarter showed complete agglutination.

DISCUSSION

In 1916, Cooledge (1) suggested the use of the agglutination test as a means of studying the presence of *Brucella abortus* in milk. From these and later experiments (2) he concluded that the udder participated in the production of agglutinins. This has since been confirmed by other workers (3), (4). The number of cases in which the cow's blood serum gives a positive titer and the milk a negative titer indicates that probably when the udder is not infected with *Brucella abortus* or other organisms, the blood agglutinins do not appear in the milk. On the other hand, it is well known that when organisms such as streptococci infect the udder and injure or destroy udder tissues, certain ingredients of the blood filter thru into the milk causing changes in its appearance, pH and composition. Thus, it might be possible for agglutinins from the blood stream of positive cows to get into the milk without a *Brucella abortus* infection of the udder.

The relation between milk agglutinin titer and presence of *Brucella abortus* in milk as demonstrated by guinea pig inoculation has been studied (5), (6), (7), (8), (9), (10) and (11). The investigators generally agree that the higher the milk titer the greater is the chance that *Brucella abortus* may be present in the milk. They do not agree on a significant titer; however, one (6) reports that milk from 69.2 percent of the cows examined, with a titer of 1 to 12.5 or over, contained the organism, while others report the milk titer of 1 to 100 or higher to be significant for the presence of the organism in milk.

Assuming that milk from a cow whose milk titer is 1 to 50 or higher may contain *Brucella abortus*, 44.4 percent of the positive cows in this study were apt to have the organism present in their milk at any time. Another 20.2 percent had milk titers of less than 1 to 50 while 35.4 percent gave negative titers in all quarters. Tweed (12) has shown the milk titer to change considerably in a relatively short time and our results corroborate this observation. In view of these facts it would seem safe to

expect that milk from any cow with a positive milk agglutination titer might contain *Brucella abortus* at any time.

SUMMARY

Agglutination tests were made on 448 samples of milk from separate quarters of the udders of 99 Bang's disease positive cows and 699 similar samples from 132 Bang's disease negative cows in the same herds.

Of the 448 samples from Bang's disease reactors 51 percent were negative, 19 percent showed partial and 30 percent complete agglutination at a dilution of 1 to 50.

The milk of 36 percent of the Bang's disease reactors gave no agglutination at 1 to 50 dilution; that of 5 percent showed partial; that of 19 percent showed complete agglutination and the milk of 40 percent showed differences in titer from different quarters of the udder.

The milk of 45 percent of the reactors, from one or more quarters of the udder, gave complete agglutination; that from 20 percent gave partial agglutination only.

When milk from only one quarter was agglutinated the agglutination was very seldom complete.

Milk samples taken at different times from the same quarter of the udder of the same cow showed considerable difference in agglutinin titer.

The percentage of agglutinations was slightly higher in milk from the hind quarters of the udder than in that from the fore quarters and complete agglutinations occurred oftener in milk from the hind quarters than in that from the fore quarters (33 and 26 percent). Difference in percentage of agglutinations in milk from right and left halves of the udder was not material (30 and 29 percent).

In only one sample out of 699 (0.14 percent) did milk from Bang's disease negative cows show any agglutination. In this instance the agglutination was only about 25 percent complete at a dilution of 1 to 50.

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APPENDIX

Table 8. Agglutinin titer of milk from separate quarters of the udders of cows that reacted positive to the agglutination blood test for Bang's disease.

Cow	Quarters of the Udder				Cow	Quarters of the Udder								
No.	LF	LH	RF	RH	No.	LF	LH	RF	RH					
Herd No. 1														
0	c	—	c	c	43	c	c	+++	c					
1	c	0	c	0	44	c	c	c	c					
2	—	—	—	—	45	c	e	c	c					
3	—	++	—	—	46	—	—	—	—					
4	c	c	c	c	47	—	—	—	—					
5	+	c	—	++	48	c	e	c	c					
6	—	—	—	—	49	+	0	+	—					
7	++	—	+	c	50	+	c	c	c					
8	—	—	—	—										
9	—	—	—	—	Herd No. 2a									
10	—	—	—	++	44	—	—	—	—					
11	c	c	c	c	45	++	+	—	+++					
12	c	c	c	c	46	—	—	c	—					
13	—	—	—	—	47	++	0	++	c					
14	—	—	—	—	48	—	++	—	++					
15	c	c	c	c	49	c	c	c	+++					
16	—	—	—	—	50	—	c	+	—					
17	—	—	—	++	52	+	—	+	+					
18	c	c	c	c	53	—	—	—	+++					
19	—	++	—	—										
20	—	—	—	—	Herd No. 2b									
21	c	c	c	c	65	+	—	—	—					
22	+	c	c	c	66	+	e	+++	c					
23	c	c	c	c	67	+++	c	c	c					
24	c	c	++	c	68	0	c	+++	c					
25	—	—	—	—	69	—	—	—	—					
26	c	c	+	c	71	—	—	—	—					
27	—	—	—	—	72	++	—	+	—					
28	c	c	c	c	73	—	—	—	—					
29	—	—	+	0	74	—	—	—	—					
30	—	—	—	0	75	—	0	—	—					
31	+	+	+	+++	76	—	+	—	—					
32	0	c	e	c	77	—	—	—	—					
33	—	—	—	—	78	—	—	—	—					
34	—	—	—	—	79	—	—	—	—					
35	+++	+	+++	—	80	+++	—	—	0					
36	+++	+++	c	—	81	c	c	+	0					
37	—	—	—	—	82	—	—	c	+++					
38	c	c	c	c	83	—	—	—	—					
39	c	c	c	c	84	—	—	—	—					
40	—	—	—	+	85	—	—	—	—					
41	c	c	c	c	86	—	—	—	—					
42	0	c	c	c	87	—	—	—	—					
					88	—	—	—	—					
					89	—	0	c	c					
					90	—	—	—	—					
					91	—	—	—	—					

Table 8—Continued

Cow	Quarters of the Udder				Cow	Quarters of the Udder			
No.	LF	LH	RF	RH	No.	LF	LH	RF	RH
Herd No. 2b—Continued									
92	0	—	0	—	23	—	—	—	—
94	++	c	+	+	24	—	—	—	—
97	c	c	c	c	27	—	++	—	—
100	—	—	—	—	—	—	—	—	—
101	—	—	—	0	—	—	—	+	—
102	—	—	—	—	—	c	c	c	c
103	+	+++	—	—	—	+	+++	+	+++
Herd No. 3									
8	—	—	—	—	29	—	—	—	—
—	—	—	—	—	38	c	c	c	c
—	—	—	—	—	44	c	c	c	c
—	—	—	—	—	57	c	c	—	++
—	—	—	—	—	—	—	c	c	—
12	—	—	—	—	—	+++	++	++	+++
14	—	—	—	—	—	++	++	+	++
—	—	—	—	—	69	+	+++	+	+
22	—	c	c	++	—	—	c	++	c
—	+	++	—	c	—	—	e	—	c
—	++	++	++	++	—	—	—	—	—

Legend. —, no agglutination; +, slight; ++ marked; +++, very marked; c, complete agglutination; 0, blind quarter; LF, left front; LH, left hind; RF, right front; RH, right hind.

